IMMUNOLOGY AND MOLECULAR BIOLOGY

A Comprehensive Screen for Chicken Proteins That Interact With Proteins Unique to Virulent Strains of Marek's Disease Virus[1]

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ABSTRACT Genetic resistance to Marek's disease (MD) has been proposed as a method to augment current vaccinal control of MD. Although it is possible to identify QTL and candidate genes that are associated with MD resistance, it is necessary to integrate functional screens with linkage analysis to confirm the identity of true MD resistance genes. To help achieve this objective, a comprehensive 2-hybrid screen was conducted using genes unique to virulent Marek's disease virus (MDV) strains. Potential MDV-host protein interactions were tested by an in vitro binding assay to confirm the initial two-hybrid results. As a result, 7 new MDV-chicken protein interactions were identified and included the chicken proteins MHC class II β (BLB) and invariant (Ii) chain (CD74), growth-related translationally controlled tumor protein (TPT1), complement component C1q-binding protein (C1QBP), retinoblastoma-binding protein 4 (RBBP4), and α-enolase (ENO1). Mapping of the encoding chicken genes suggests that *BLB*, the gene for MHC class II β chain, is a positional candidate gene. In addition, the known functions of the chicken proteins suggest mechanisms that MDV might use to evade the chicken immune system and alter host gene regulation. Taken together, our results indicate that integrated genomic methods provide a powerful strategy to gain insights on complex biological processes and yield a manageable number of genes and pathways for further characterization.

(*Key words*: gene mapping, genetic resistance, Marek's disease, protein interactions, two-hybrid screen)

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INTRODUCTION

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by the MD virus (MDV), a herpesvirus. As MDV persists and is ubiquitous in poultry houses, all commercial chicks are exposed at 1 d of age. Susceptible chickens develop enlarged nerves and lymphomas in visceral tissues, which leads to paralysis, blindness, and eventually death. Vaccines are able to protect against MD, but they do not prevent MDV replication and spread. With the periodic emergence of more virulent MDV strains (Witter, 1997), MD control requires either the development of new and more effective vaccines or the introduction of alternative measures to augment vaccinal control.

Genetic resistance to MD has been proposed as one such alternative method. MD resistance has been known to exist for 60+ yr, and birds can be selected for higher levels of resistance (reviewed in Bacon et al., 2001). Genetic resistance to MD is complex and controlled by multiple genes. It is fairly well established that specific haplotypes of the MHC or, as it is known in the chicken, the B complex are associated with MD resistance or susceptibility. QTL conferring resistance to MD have also been identified (Bumstead, 1998; Vallejo et al., 1998; Yonash et al., 1999). Although this knowledge has demonstrated that there are heritable units that allow for selection, the QTL intervals are not sufficiently resolved to reveal the causative gene or allele. This lack of resolution makes it difficult to select improved MD resistance based on genetic markers alone. This challenge is not unique to MD genetic resistance and, in fact, probably represents one of the greatest challenges facing all scientists analyzing complex genetic traits (Dodgson et al., 2000; Page et al., 2003).

Confronted with this problem, we have been integrating functional genomic screens with genetic association studies to reveal positional candidate genes that confer resistance to MD. In other words, gene profiling

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Abbreviation Key: C1QBP = C1q-binding protein; EBV = Epstein-Barr virus; EBNA1 = Epstein-Barr nuclear antigen-1; ENO1 = α-enolase; GST = glutathione S-transferase; HSV1 = herpes simplex virus type 1; iI = MHC class II invariant; MD = Marek's disease; MDV = Marek's disease virus; SNP = single nucleotide polymorphism.

by DNA microarrays, 2-hybrid assays, or both identify those candidate genes with functional properties (gene expression, protein association) related to MDV infection. Those candidates can be evaluated (subsequently or in parallel) by genetic mapping or association studies. Because the approaches are independent of one another, candidates identified by 2 or more methods can be pursued with much greater confidence. This strategy has successfully identified growth hormone (*GH1*) and lymphocyte antigen 6 complex, locus E (*LY6E*; also known as stem cell antigen 2 or *SCA2*) as MD resistance genes (Liu et al., 2001b, 2003). Both genes encode proteins that interact with MDV proteins, their mRNA are differentially expressed between MD-resistant and susceptible lines, they encode alleles significantly associated with one or more MD traits, and their known gene functions appear to be consistent with our knowledge of MDV biology.

The objective of this study was to extend our 2-hybrid assay of MDV-chicken protein interactions. In particular, we performed a comprehensive screen of all the MDV genes that are believed to be unique to virulent MDV strains. The identified chicken proteins should provide insight on how MDV might alter normal host functions. Furthermore, genetic mapping of the encoding genes should determine if one or more of these genes are positional candidate genes for MD resistance based on their location within an MD QTL interval.

MATERIALS AND METHODS

Escherichia coli 2-Hybrid Assay

The screen for MDV-chicken protein-protein interactions was performed with a 2-hybrid system[5] according to the instructions provided by the manufacturer. MDV proteins (baits) were selected based on the following criteria: (1) the encoding gene was present in the GA strain (GenBank accession no. AF147806 and L22174) and Md5 strain (GenBank accession no. AF243438) sequences, (2) the encoding gene was not present in MDV serotype 2 (GenBank accession no. AB049735) or herpesvirus of turkey (HVT) (GenBank no. AF282130 and AF291866) sequences, and (3) the MDV protein has not been the subject of significant previous functional characterization [i.e., R-LORF2 (vIL8), R-LORF7 (Meq), R-LORF14 (pp24), and R-LORF14a (pp38)]. US1 was also included due to its potential role in attenuation (Jones et al., 1996).

The MDV genes were amplified by PCR using DNA isolated from chicken embryo fibroblasts infected with MDV strain Md5 and the primers listed in Table 1 as described in Liu and Cheng (2003). All the forward primers generated an *Eco*RI restriction enzyme site except those for R-LORF8, R-LORF13a, and LORF4, which created *Not*I, *Bam*HI, and *Bam*HI sites, respectively. All reverse primers included an *Xho*I site except for R-LORF1, R-LORF8, and LORF8, which incorporated *Bam*HI sites. The resulting amplicons were digested with the appropriate restriction enzymes and were cloned in-frame into the identical restriction enzyme sites of a modified pBT vector; a frame flexible linker sequence [$(Gly_4Ser)_3$] was placed between the insert and the λ repressor as recommended by the manufacturer. A chicken cDNA library derived from activated splenic T cells previously described (Liu et al., 2001b) was fused with the pTRG target vector. The MDV-containing pBT plasmid and the cDNA library were cotransformed into competent *E. coli* cells, and colonies were selected for carbenicillin resistance and the presence of β-galactosidase activity. Positive transformants were confirmed by selection on new plates. Plasmids were isolated and subjected to DNA sequencing by dye-terminator fluorescence sequencing on an automatic DNA sequencer[6] using the pTRG sequencing primer described in the screening kit. Sequence data were queried for open reading frames and to public databases using the BLAST program (http://www.ncbi.nlm.nih.gov/blast).

In Vitro Binding Assay

To confirm the interaction of a protein that was detected by the bacterial 2-hybrid system, the cDNA was amplified and cloned in frame into the pGEX-5X vector[7] to produce a glutathione S-transferase (GST)-chicken fusion protein. The fusion protein was adsorbed on glutathione Sepharose 4B beads[7] and then used in the in vitro binding assay. The corresponding MDV gene was amplified and cloned in frame into the pET28a expression plasmid, and protein was synthesized in vitro using the Single Tube Protein System 3 (STP3).[8] One microgram of the expression plasmids was used as template for a coupled T7-directed in vitro transcription-translation reaction in the presence of 40 μCi [^{35}S]-methionine. Ten microliters of the reaction was incubated with Sepharose bead-adsorbed GST fusion protein or GST alone for in vitro protein-binding assays. After serial washing with 1% Triton X-100 in PBS, bound protein was eluted with 30 μl of elution buffer (10 m*M* reduced glutathione in 50 m*M* Tris-HCl, pH 8.0). All samples were subjected to 10% SDS-PAGE analysis followed by autoradiography.

Linkage Analysis

Mapping of relevant chicken genes was performed essentially as described by Smith and Cheng (1998) and Liu and Cheng (2003). Briefly, primers were designed (Table 2) to amplify an intron (*TPT1*) or the 3′ untranslated region (*CD74* and *RBBP4*) of each chicken gene; for *C1QBP*, a MegaBlast database search using GenBank accession no. AB029946 as the query retrieved the Trace Archive sequence 261543180, which contains part of an intron. Amplicons were generated using DNA from Red Jungle Fowl (UCD-001) or White Leghorn (UCD-003), the par-

[5]BacterioMatch, Stratagene, La Jolla, CA.
[6]ABI 3100, Applied Biosystems, Foster City, CA.
[7]Amersham Biosciences, Piscataway, NJ.
[8]Novagen, Madison, WI.

TABLE 1. Marek's disease virus (MDV) proteins used as bait in a 2-hybrid screen and the primers used to amplify the corresponding gene

MDV ORF[1]	Forward primer[2]	Reverse primer[2]
R-LORF1	gagaattcgATGACCCGGGGGCATCGCAC	ccggatccTTACTCGCTGACTTTCAGCGGGC
R-LORF3	acgaattcgATGACAACCCCATATTTTGGC	ggctcgagCTATACTGATGTAACCAGTACTC
R-LORF4	cggaattcgATGCGCCAGTTATGCATGACG	ggctcgagCTATAGCATCGAAACACTAAAATG
R-LORF6	ccgaattcgATGAACCTAACGCTCCACATTG	gactcgagTTATATATAACTAGGGGAGAAG
R-LORF8	cagcggccgcATGACTATCGTCTTTATATCAC	tgggatccTCACATTTTTAAGTTGTATGTAG
R-LORF9	gtgaattcgATGTGGAACACGATTGGCCG	gcctcgagTTATCGATAATCGGCTCCGATCC
R-LORF10	gggaattcgATGTGCAAACAGCGACTCATTATC	gcctcgagCTACCATCCGTGTTGATTACAGC
R-LORF11	tcgaattcgATGTTACATATCTATAACTTGATT	gactcgagTTATACTTTACCTGCTTTTGC
R-LORF12	tagaattcgATGTGTATGCAAATGAGCAG	tactcgagTTATTACTTATTTGATGAAGGGAG
R-LORF13a	ctggatccATGACCCACAACCGCGATTTG	ccctcgagTCACCCTTTATTGGAATAGCC
MDV004	cggaattcgATGTGGGGGAGATGGGGTAAA	cactcgagTCACCGGATGAACCTAACGC
LORF1	gcggccgcGATGGTGAATAGACGCAACTAT	ggatccTTATTGGTTCGCAGTGCGAACGCTGAC
LORF4	ttggatccATGCAACCCGATCCGCGATTTC	atctcgagTTATAGAGTACTCGTGCATCTTTC
LORF5	tcgaattcgATGAGTAAACCATATCATGCTC	aactcgagTTATCCTATGACAACAGAAGTGC
LORF6	tcgaattcgATGACTGTATCTAATCCATACGC	gcctcgagTCAAATATCCGAATTTAACTTCA
LORF8	gggaattcgATGGTGGGTAGTATACAGGTAG	ttggatccCTATGACAAAAGAGTTGCACGG
LORF10	gggaattcgATGGGCATTATTTTTTCCAACC	gcctcgagTCAATCTACTGTTGTTGGTCC
LORF12	cgggatccATGTTGGAGGCGGAGATTAAGC	tactcgagTTATGTTTCTGTGATAATAGTTAC
SORF4	ttgaattcgATGGCACCTTCGGGACCTACGC	ttctcgagTTAGAAAAATGAGAATGAAAT
US1	gggaattcgATGAGTCGTGATCGAGATCGAG	ctctcgagTTAATGCAATTTACTGTCTACCG

[1]Nomenclature of open reading frame (ORF) based on Lee et al. (2000) and Lupiani et al. (2001).

[2]Capitalized letters match the sequence to the target gene, whereas those in lowercase are designed to generate a unique restriction enzyme site for cloning into the pBT vector (Stratagene, La Jolla, CA).

TABLE 2. Primers used to detect and genotype single nucleotide polymorphisms (SNP) in chicken genes for genetic mapping

Gene	Primers used for screening	SNP[1]	Primers used for genotyping[2]
C1QBP	TAGAATATCCCACGCTGCTG CATCGGCAAATGTGTTATCC	C/T (526)	Same as those used for screening
CD74	GACATGGTGAAAGCCAAGTAG CAGGGGTCAGTTAAGCTCGGG	A/G (1008) GTC/CCG (1070–1072)	TCAGTTACATCCCTTCCCCTA CAGTAAATAAAGCTGTCAGAC
TPT1	CCATGATCATCTACCGGGAC TTAACATTTCTCAATCTC	C/T (363)	TGAGGATATTAGTGGCCTTGA TTAACATCCGTAACTC[G]AGCT
RBBP4	CCAGAAGGTCAAGGATCTTAG GCAGGTACAGATTAACTAAAG	AAG/A:G (1529–1530) A/G (1598)	CCCTAAGAAAAGGGCTTTTAaG GCTAAAATGGGATGGCCAGCT

[1]The base(s) represent UCD-001 (Jungle Fowl) and UCD-003 (White Leghorn) allele sequence, respectively. The number(s) in the parentheses are the position(s) of the sequence based on GenBank accession no. 261543180 (Trace Archive), AJ292038, AY383617, and AF097750 for *C1QBP*, *CD74*, *TPT1*, and *RBBP4*, respectively.

[2]Base(s) underlined are specific to the UCD-001 allele. The base in a box represents an intentional mismatch used to create a restriction enzyme site with the UCD-001 SNP allele. The base that is not capitalized is a 1-bp insertion present in the UCD-001 allele but not in UCD-003 allele.

TABLE 3. Marek's disease virus-chicken protein interactions confirmed by in vitro binding assay and their genetic map location

MDV protein		Interacting chicken protein			
Encoding ORF[1]	Protein size[2]	Name	Gene symbol	Protein size[2]	Map location[3]
R-LORF8	135	complement component C1q-binding protein	*C1QBP*	208	GGA19 (9)
R-LORF10	95	MHC class II invariant chain	*CD74*	223	GGA13 (55)
R-LORF12	115	growth-related translationally-controlled tumor protein	*TPT1*	173	GGA01 (474)
R-LORF13	104	complement component C1q-binding protein	*C1QBP*	208	GGA19 (9)
LORF4	142	MHC class II β chain	*BLB*	263	GGA16 (0)
MDV004	136	α-enolase	*ENO1*	434	E54 (30)
SORF2	179	growth hormone[4]	*GH*	216	GGA27 (5)
US1	179	retinoblastoma-binding protein 4	*RBBP4*	425	GGA23 (20+)
US10	213	stem cell antigen 2[5]/lymphocyte antigen 6 complex, locus E	*SCA2/LY6E*	127	GGA02 (415)

[1]ORF = open reading frame. Nomenclature of ORFs is based on Lee et al. (2000).

[2]Predicted number of amino acids.

[3]Chromosome assignment based on the consensus map described in Schmidt et al. (2000). The number in the parentheses is the estimated position in cM.

[4]This interaction was previously described in Liu et al. (2001b).

[5]This interaction was previously described in Liu et al. (2003).

ents of the East Lansing reference panel (Crittenden et al., 1993). The products were cloned into a TA cloning vector;[9] the resulting plasmids were isolated, and their DNA sequences were determined as described above. Single nucleotide polymorphisms (SNP) between Red Jungle Fowl and White Leghorn alleles were identified. For *C1QBP*, one SNP generated a *Tsp*45I restriction enzyme site with the Red Jungle Fowl allele only. For *CD74* and *RBBP4*, more than one SNP was detected, which enabled the generation of primers that were highly specific to the Red Jungle Fowl allele (Table 2). For *TPT1* where a single SNP was detected but did not create a polymorphic restriction site in either allele, mismatched primers were used that created a *Sac*I restriction enzymes site with the Red Jungle Fowl SNP allele only (Table 2). Genotype analysis was done by PCR amplification, restriction enzyme digestion (when necessary), followed by agarose gel electrophoresis. Multipoint linkage analysis of the genotypes through Map Manager, version 2.6.5 (Manly and Olson, 1999) allowed for the optimal placement of each gene in the existing data set, which contains ~1,250 loci.

RESULTS AND DISCUSSION

Advances in genomic technologies enhance our ability to gain insights on complex biological processes such as host-pathogen interactions and genetic resistance to disease. With the development of commercial 2-hybrid assays based in *E. coli*, combined with the availability of the complete MDV genome sequence (Lee et al., 2000; Tulman et al., 2000), it became feasible to conduct the first comprehensive screen of MDV-chicken protein-protein interactions. Rather than screening all MDV proteins, we chose to concentrate on proteins unique to serotype 1 (virulent) MDV strains compared with serotype 2 strains and herpesvirus of turkey. We anticipated that virulence and other distinct properties of serotype 1 MDV strains are primarily due to genes and gene products found only in these strains. US1 was also screened because of its potential role in attenuation in the RM1 strain (Jones et al., 1996). After eliminating some MDV proteins that have already been examined extensively, 20 MDV proteins were selected and characterized (see Table 1).

For each MDV gene (bait), at least 500,000 transformants were screened to ensure reasonable coverage of the cDNA library. Typically, 5 to 20 positive colonies were identified although the range was from 1 to over 50. The majority of these chicken genes (prey) were eliminated after DNA sequencing as only 0 to 4 plasmids were found to contain significant open reading frames per MDV gene. Because of the tendency of the 2-hybrid screen to produce false positives, a widely recognized problem, it is necessary to have an independent confirmation step. The in vitro binding was chosen, which biochemically detects protein-protein interactions. As part of the procedure, the chicken cDNA is expressed and purified as GST fusion protein, which is the most technically difficult and rate-limiting step in the entire process. Consequently, only chicken genes that have been previously identified or have significant homology to identified genes in other species were examined. A successful interaction is shown in Figure 1, where R-LORF10 was retained with the GST-CD74 fusion protein but was not retained with GST protein alone (control). This result also indicates that the interaction between R-LORF10 and CD74 is a direct and specific protein-protein interaction and does not require other intermediary factors (e.g., bacterial proteins). In the end, 7 new MDV-host protein-protein interactions were identified and confirmed (Table 3).

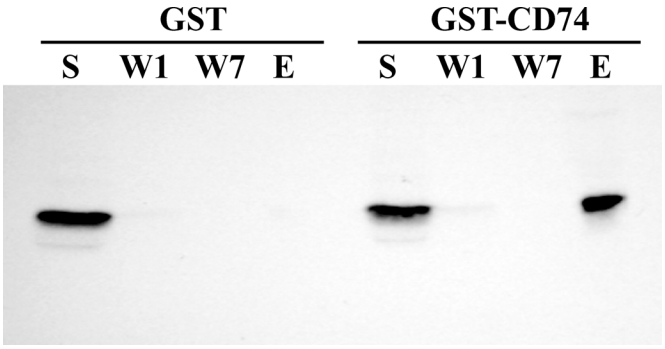

FIGURE 1. R-LORF10 and CD74 (MHC class II invariant chain) interact in vitro. Supernatant (S) shows the input of ^{35}S-labeled R-LORF10 to the affinity columns. After 1 and 7 washes (W1 and W7, respectively), R-LORF10 is retained and eluted (E) with the glutathione S-transferase (GST)-CD74 fusion protein but not with GST alone.

The R-LORF8 and R-LORF13, proteins of unknown function, interact with complement component C1q-binding protein (C1QBP; Ghebrehiwet et al., 1994), which is also known as the p32 subunit of splicing factor ASF/SF2 (SF2P32 or P32; Krainer et al., 1991) and hyaluronic acid-binding protein 1 (Deb and Datta, 1996). C1QBP interacts with other viral proteins. Those interacting proteins encoded by herpesviruses include herpes simplex virus type 1 (HSV1) ICP27, which is involved in transcriptional and posttranscriptional regulation and is essential for viral replication (Bryant et al., 2000); HSV1 ORF P, which is expressed during HSV1 latency (Bruni and Roizman, 1996); herpesvirus saimiri ORF 73, which is expressed during herpesvirus saimiri latency (Hall et al., 2002); and Epstein-Barr virus (EBV) nuclear antigen-1 (EBNA1), which binds to the EBV origin of replication and is required for the maintenance of the viral genome during latency (Wang et al., 1997; Chen et al., 1998; Van Scoy et al., 2000). The EBNA1-C1QBP interaction supports our R-LORF13-C1QBP interaction as R-LORF13 exhibits significant homology to EBNA1 (Lupiani et al., 2001).

The R-LORF10 and LORF4, proteins with no known function, interact with MHC class II invariant chain (Ii, also called γ-chain or CD74) and β-chain, respectively. The mature MHC class II molecules that present antigenic

[9]Invitrogen, Carlsbad, CA.

peptides to CD4+ T cells are a heterodimer of α- and β-chains. However, newly synthesized MHC class II molecules consist of α-, β-, and Ii-chains. Ii-chain plays a critical role in class II formation, transport out of the endoplasmic reticulum and through the Golgi complex, and antigen loading (reviewed by Stumptner-Cuvelette and Benaroch, 2002; Matza et al., 2003).

Many viruses with large genomes, such as herpesviruses, encode proteins that target the MHC class I and II antigen-presenting systems to escape detection and elimination by the immune system of the host (reviewed by Vossen et al., 2002; Hegde et al., 2003). For example, with respect to class II, HSV1 glycoprotein B down-regulates class II expression by binding class II α-chain and reducing expression of Ii-chain (Neumann et al., 2003). Human cytomegalovirus US2 induces degradation of class II α-chain (Tomazin et al., 1999), whereas US3 inhibits the association of class II with the Ii-chain (Hegde et al., 2002). All of these viral proteins effectively reduce the cell surface expression of MHC class II molecules and inhibit antigen presentation to CD4+ T cells. The EBV glycoprotein 42, which is encoded by *BZLF2*, binds and uses class II as a cofactor for viral infection of B cells (Li et al., 1997). The interaction of MDV proteins with MHC class II components would suggest that MDV also influences class II cell surface expression (Gimeno et al., 2001) as is the case for class I (Hunt et al., 2001; Levy et al., 2003).

Infected cell protein 22 (ICP22), the gene product of MDV *US1*, transactivates other viral genes, probably in conjunction with ICP4 (Kato et al., 2002). ICP22 interacts with retinoblastoma-binding protein 4 (RBBP4), which is also known as the 48-kDa subunit of chromatin assembly factor-1 (CAF-1p48). RBBP4, in turn, interacts with histone deacetylase-2 (HDAC2) (Ahmad et al., 1999). As modifications in chromatin influence haematopoietic cell fate (reviewed by Georgopoulos, 2002), one could speculate that MDV may regulate host transcription though ICP22 binding to RBBP4 to create an environment more suitable for viral replication and spread. The MDV protein interaction with RBBP4 is also intriguing because of its potential involvement in well-recognized and important cellular processes, especially with respect to deregulation of the retinoblastoma pathway, a "hallmark of cancer" (Hanahan and Weinberg, 2000).

The MDV R-LORF12, a hypothetical protein of unknown function, interacts with the p23 member of the translationally controlled tumor protein (TCTP) family encoded by the *TPT1* gene. TCTP are ubiquitous, abundant, and highly conserved proteins across eukaryotes. Originally thought to be tumor specific and controlled at the translational level (Gross et al., 1989), it is clear that TCTP are expressed in a wide variety of tissues (e.g., erythrocytes; Sanchez et al., 1997) and can be transcriptionally regulated (e.g., Xu et al., 1999). Described as growth-related proteins, TCTP have been reported to have a variety of functions including (1) involvement in IgE-dependent histamine and interleukin-4 release (MacDonald et al., 1995; Schroeder et al., 1996), (2) acting as a B-cell growth factor (Kang et al., 2001), and (3) regulating the cell cycle by interaction with tubulin binding protein (Gachet et al., 1999). *TPT1* has also been implicated with PKR, a double-stranded RNA-dependent protein kinase. PKR is activated by double-stranded RNA that are produced during viral replication and gene expression as part of the interferon-induced antiviral system of the host. Some viruses express small RNA that bind to PKR and prevent its activation (e.g., Gunnery et al., 1990; Henry et al., 1994; Robertson et al., 1996). *TPT1* mRNA has been shown to activate PKR as well as be translationally regulated by PKR.

The MDV004, a 23-kDa protein detected in the nuclear fraction of MDV-infected cells (Peng and Shirazi, 1996), interacts with α-enolase (ENO1). ENO1 converts 2-phosphoglycerate to phosphoenolpyruvate during glycolysis. An abundant cytosolic protein, ENO1 is also found on the surface of hematopoietic cells (e.g., T and B cells) where its expression is modulated by physiological conditions (reviewed by Pancholi, 2001). Because of its ability to act as a plasminogen receptor and the direct correlation between ENO1 expression and tumor progression, ENO1 has been suggested as a diagnostic marker for many cancers and neurological diseases (Eriksson et al., 2000). Adding to the interest in ENO1, an alternative initiation product of ENO1 mRNA is the myc-binding protein (MBP-1), which can suppress tumors by binding the *c-myc* promoter (Subramanian and Miller, 2000).

Although our MDV-chicken protein interactions have been confirmed by in vitro binding, and for several cases there is supporting literature, all of the interactions have to be viewed as tentative until confirmed in the environment of the natural host, which is underway. For example, although an MDV gene may be predicted, it may be possible that the corresponding protein is not expressed (Murphy et al., 2003). Conversely, the 2-hybrid screen favors strong interactions and produces false negatives so there may well be additional MDV-host protein interactions that were missed.

Besides finding interacting partners for MDV proteins and insights on potential MDV-host regulatory pathways, 2-hybrid screens serve to identify genes that may encode MD resistance alleles. Genes so identified are mapped to determine if they are located within a MD QTL interval. If the gene of interest is located in an area that was not well surveyed in previous whole genome scans, then it is possible to conduct an association tests between the gene and MD traits after the fact. Thus far, this integrative approach has successfully revealed *GH1* and *LY6E* as MD resistance genes based on the criteria that the genes are associated with MD traits, their proteins interact with an MDV protein, and they have a known function that is consistent with MD involvement (Liu et al., 2001b, 2003). Differential gene expression between MD-resistant and susceptible strains also reinforces the conclusion that these 2 genes are associated with MD resistance (Liu et al., 2001a).

Mapping of these 6 new candidate genes suggests that *BLB* is another positional candidate gene because the MHC locus is associated with MD resistance (reviewed by Bacon et al., 2001). It is not possible to determine which of the 19 genes contained in the chicken MHC contributes to the

MD resistance phenotype because all MHC genes are inherited as a single haplotype. Our observation that the class II β-chain interacts with MDV LORF4 suggests that *BLB* is at least one of the responsible genes.

In addition to comparing map locations of candidate genes with those of previously identified QTL, this process also builds the chicken-human comparative map. With the exception of *RBBP4*, all 4 genes mapped to the location predicted by the position of their human homologues in that map (Schmidt et al., 2000); *ENO1* was previously mapped to linkage group E54 (Smith et al., 1997), which exhibits conserved synteny to HSA1. Specifically the map location of *C1QBP*, *CD74*, and *TPT1* add to the conserved synteny previously observed between GGA19 and HSA17, GGA13 and HSA5, and GGA01 and HSA13. *RBBP4* is located on GGA23 with the corresponding human homolog placed on the cytogenetic map at 1p34. This region does not exhibit conserved synteny because human homologs that are linked to *RBBP4* are found on GGA05, GGA08, and linkage groups E54 and C15 (Schmid et al., 2000); therefore, it is not possible to accurately predict where chicken homologs should be found.

In conclusion, functional genomic screens such as 2-hybrid assays and DNA microarray analyses provide considerable insight into complex biological processes such host-pathogen interactions. When integrated with genetic association studies, these combined approaches identify positional candidate genes that can be characterized further using more traditional approaches. Future experimental and in silico methods will undoubtedly facilitate drawing accurate correlations between genotype and phenotype to provide information that will enhance poultry breeding and management strategies dealing with MD.

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